PATENT COOPERATION TREATY

From the
INTERNATIONAL SEARCHING AUTHORITY

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PCT

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

(PCT Rule 43*bis*.1)

Date of mailing (*day/month/year*)	30 JAN 2009

Applicant's or agent's file reference 78340-A-PCT	FOR FURTHER ACTION See paragraph 2 below	
International application No. PCT/US 08/11913	International filing date (*day/month/year*) 17 October 2008 (17.10.2008)	Priority date (*day/month/year*) 19 October 2007 (19.10.2007)
International Patent Classification (IPC) or both national classification and IPC IPC(8) - C12Q 1/68; C12N 9/22; A61K 31/70 (2009.01) USPC - 435/6, 199; 514/44		
Applicant THE TRUSTEES OF COLUMBIA UNIVERSITY IN THE CITY OF NEW YORK		

1. This opinion contains indications relating to the following items:

- ☒ Box No. I Basis of the opinion
- ☐ Box No. II Priority
- ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- ☐ Box No. IV Lack of unity of invention
- ☒ Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- ☐ Box No. VI Certain documents cited
- ☐ Box No. VII Certain defects in the international application
- ☐ Box No. VIII Certain observations on the international application

2. **FURTHER ACTION**

If a demand for international preliminary examination is made, this opinion will be considered to be a written opinion of the International Preliminary Examining Authority ("IPEA") except that this does not apply where the applicant chooses an Authority other than this one to be the IPEA and the chosen IPEA has notified the International Bureau under Rule 66.1*bis*(b) that written opinions of this International Searching Authority will not be so considered.

If this opinion is, as provided above, considered to be a written opinion of the IPEA, the applicant is invited to submit to the IPEA a written reply together, where appropriate, with amendments, before the expiration of 3 months from the date of mailing of Form PCT/ISA/220 or before the expiration of 22 months from the priority date, whichever expires later.

For further options, see Form PCT/ISA/220.

3. For further details, see notes to Form PCT/ISA/220.

Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Date of completion of this opinion 15 January 2009 (15.01.2009)	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/237 (cover sheet) (April 2007)

WRITTEN OPINION OF THE INTERNATIONAL SEARCHING AUTHORITY

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Box No. I Basis of this opinion

1. With regard to the **language**, this opinion has been established on the basis of:
   - [x] the international application in the language in which it was filed.
   - [ ] a translation of the international application into ____________ which is the language of a translation furnished for the purposes of international search (Rules 12.3(a) and 23.1(b)).

2. [ ] This opinion has been established taking into account the **rectification of an obvious mistake** authorized by or notified to this Authority under Rule 91 (Rule 43*bis*.1(a))

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, this opinion has been established on the basis of:

   a. type of material
      - [ ] a sequence listing
      - [ ] table(s) related to the sequence listing

   b. format of material
      - [ ] on paper
      - [ ] in electronic form

   c. time of filing/furnishing
      - [ ] contained in the international application as filed
      - [ ] filed together with the international application in electronic form
      - [ ] furnished subsequently to this Authority for the purposes of search

4. [ ] In addition, in the case that more than one version or copy of a sequence listing and/or table(s) relating thereto has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that in the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

5. Additional comments:

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Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of

☐ the entire international application

☒ claims Nos. 5-8, 10-12, 17-20, 22-24 and 27

because:

☐ the said international application, or the said claims Nos. ______ relate to the following subject matter which does not require an international search (*specify*):

☒ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. see below are so unclear that no meaningful opinion could be formed (*specify*):

Claims 5-8, 10-12, 17-20 and 22-24 are improper multiple dependent claims because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Claim 27 is an improper omnibus claim, not drafted in accordance with Rule 6.2.

☐ the claims, or said claims Nos. ______ are so inadequately supported by the description that no meaningful opinion could be formed (*specify*):

☒ no international search report has been established for said claims Nos. 5-8, 10-12, 17-20, 22-24 and 27

☐ a meaningful opinion could not be formed without the sequence listing; the applicant did not, within the prescribed time limit:

- ☐ furnish a sequence listing on paper complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ furnish a sequence listing in electronic form complying with the standard provided for in Annex C of the Administrative Instructions, and such listing was not available to the International Searching Authority in a form and manner acceptable to it.
- ☐ pay the required late furnishing fee for the furnishing of a sequence listing in response to an invitation under Rule 13*ter*.1(a) or (b).

☐ a meaningful opinion could not be formed without the tables related to the sequence listings; the applicant did not, within the prescribed time limit, furnish such tables in electronic form complying with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions, and such tables were not available to the International Searching Authority in a form and manner acceptable to it.

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in electronic form only, do not comply with the technical requirements provided for in Annex C-*bis* of the Administrative Instructions.

☐ See Supplemental Box for further details.

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Box No. V Reasoned statement under Rule 43*bis*.1(a)(i) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Claims	1-4, 9, 13-16, 21, 25-26, 35-59	YES
	Claims	28-34	NO
Inventive step (IS)	Claims	none	YES
	Claims	1-4, 9, 13-16, 21, 25-26, 28-59	NO
Industrial applicability (IA)	Claims	1-4, 9, 13-16, 21, 25-26, 28-59	YES
	Claims	none	NO

2. Citations and explanations:

Claims 28-34 lack novelty under PCT Article 33(2) as being anticipated by US 2007/0166705 A1 Milton et al. (hereinafter 'Milton')

As per Claim 28, Milton teaches a compound having the structure: (structure omitted, see applicant's Claim 28), wherein R' is a cleavable chemical group (para [0145], cleavable linker, Figure 2, para [0119] 3' O chemical group, Figure 3)

As per Claim 29, Milton further teaches the compound of claim 28, wherein R' is a nitrobenzyl group, an allyl group or a methylazido group (para [0087], nitrobenzyl Figure 3, para [0040], allyl, para [0068], Figure 3, methylazido group)

As per Claim 30, Milton further teaches the compound of claim 28, wherein the base has a detectable marker cleavably linked thereto (para [0025], Figure 1).

As per Claim 31, Milton teaches a deoxyribonucleic acid having attached at a 3' end thereof, by a phosphodiester bond, a compound having the structure:(structure omitted, see applicant's Claim 31), wherein the O atom labeled a is the 3' O atom of the deoxyribonucleic acid, the wavy line represents the remainder of the deoxyribonucleic acid that is 5' relative to the 3' O, and wherein R' is a cleavable chemical group (para [0142]-[0145], nucleotide is incorporated, detected and the the 3' O blocking group is cleaved).

As per Claim 32, Milton further teaches the deoxyribonucleic acid of claim 31, wherein R' is a nitrobenzyl group, an allyl group or a methylazido group (para [0087], nitrobenzyl Figure 3, para [0040], allyl, para [0068, Figure 3], methylazido group).

As per Claim 33, Milton further teaches the deoxyribonucleic acid of claim 32, attached to a solid surface (para [0137]).

As per Claim 34, Milton further teaches a kit for sequencing a nucleic acid is provided comprising detectably-labeled dideoxynucleotide riphosphate analogues and the dITP analogue of claim 31 and instructions for use in sequencing (para [0059], kit, para [0095], nucleotides embodied in invention).

Claims 1-4, 9, 13-16, 21, 25-26 and 35-59 lack an inventive step under PCT Article 33(3) as being obvious over US 7,078,499 B2 to Odedra et al. (hereinafter 'Odedra') in view of Milton.

As per Claim 1, Odedra teaches a method for determining the identity of each of a series of consecutive nucleotide residues in a nucleic acid comprising: a) contacting a plurality of the nucleic acids with (i) a dideoxynucleotide triphosphate (ddNTP) analogue having the structure: (structure omitted, see applicant's Claim 1) wherein F is a fluorophore, L is a cleavable linker molecule and the base is adenine, guanine, cytosine, uracil or thymine (col 3, ln 1-20, Z can be H, Y is a cleavable linker, R2 is a fluorophore), and wherein each base has a different fluorophore attached (col 5, ln 62-65), and wherein the fluorophore attached to each type of base differs in its excitation or emission spectra from the fluorophores attached to the other types of bases (col 6, ln 1-5), but does not teach (ii) a deoxynucleotide triphosphate having the structure: (structure omitted, see applicant's Claim 1) (dNTP) analogue wherein B is a base and is adenine, guanine, cytosine, uracil, thymine or an inosine, and wherein R' is a cleavable chemical group, (iii) a nucleic acid polymerase and (iv) at least two primers each of which hybridizes with a separate nucleic acid of the plurality of nucleic acids, under conditions permitting a ddNTP analogue that is complementary to the consecutive nucleotide residue to be identified to form a phosphodiester bond with the 3' end of one of the primers and a dNTP analogue that is complementary to the consecutive nucleotide residue to be identified to form a phosphodiester bond with the 3' end of another of the primers; b) identifying the fluorophore of the ddNTP analogue which has formed the phosphodiester bond thereby identifying the identify of the consecutive nucleotide; c) cleaving the fluorophore from the ddNTP analogue which has formed the phosphodiester bond and cleaving the cleavable chemical group from the dNTP which has formed the phosphodiester bond; d) iteratively repeating steps a) through c) for each of the consecutive nucleotide residues to be identified until the final consecutive nucleotide residue is to be identified; e) repeating steps a) and b) to identify the final consecutive nucleotide residue, thereby determining the identity of each of the series of consecutive nucleotide residues in the nucleic acid.

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Supplemental Box

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Box No. V 2. Citations and explanations:

(claim 1 cont'd) While does not teach a method for determining the identity of each of a series of consecutive nucleotide residues in a nucleic acid comprising: a) contacting a plurality of the nucleic acids with (i) a dideoxynucleotide triphosphate (ddNTP) analogue having the structure: (structure omitted, see applicant's Claim 1) wherein F is a fluorophore, L is a cleavable linker molecule and the base is adenine, guanine, cytosine, uracil or thymine, and wherein each base has a different fluorophore attached, and wherein the fluorophore attached to each type of base differs in its excitation or emission spectra from the fluorophores attached to the other types of bases, Milton does further teach a deoxynucleotide triphosphate having the structure: (structure omitted, see applicant's Claim 1) (dNTP) analogue wherein B is a base and is adenine, guanine, cytosine, uracil, thymine or an inosine, and wherein R' is a cleavable chemical group (para [0147], azidomethyl group is applicant's R', para [0118], 32P label), (iii) a nucleic acid polymerase (para [0045]). Neither Odedra nor Milton individually teach a method for determining the identity of each of a series of consecutive nucleotide residues in a nucleic acid comprising: (iv) at least two primers each of which hybridizes with a separate nucleic acid of the plurality of nucleic acids, under conditions permitting a ddNTP analogue that is complementary to the consecutive nucleotide residue to be identified to form a phosphodiester bond with the 3' end of one of the primers and a dNTP analogue that is complementary to the consecutive nucleotide residue to be identified to form a phosphodiester bond with the 3' end of another of the primers; b) identifying the fluorophore of the ddNTP analogue which has formed the phosphodiester bond thereby identifying the identify of the consecutive nucleotide; c) cleaving the fluorophore from the ddNTP analogue which has formed the phosphodiester bond and cleaving the cleavable chemical group from the dNTP which has formed the phosphodiester bond; d) iteratively repeating steps a) through c) for each of the consecutive nucleotide residues to be identified until the final consecutive nucleotide residue is to be identified; e) repeating steps a) and b) to identify the final consecutive nucleotide residue, thereby determining the identity of each of the series of consecutive nucleotide residues in the nucleic acid. However, Milton contemplates the use of a specific embodiment of his invention in Sanger or Sanger like reactions (para [0115], ddNTP approach) in which the 3' O is blocked using applicant's R' blocking group as well as having a fluorophore attached to the base (applicant has fluorophore attached to a separate, ddNTP nucleotide) while incorporating sequencing by synthesis approach (each added nucleotide is detected). In this context, it would have been obvious to one of ordinary skill in the art to apply the teachings of Odedra, namely the use of a deoxyribonucleotide having a cleavable linker attached to reporter group bound to the nitrogen base in which the 3' OH is either blocked or replaced by a H (making it a ddNTP) to the teachings of Milton, namely, the use of a deoxyribonucleotide having a cleavable 3' azidomethyl blocking group to generate the method described in Claim 1, because the utility of employing the Sanger approach without the expense of of a gel electrophoresis would be of great benefit..

As per Claim 2, Milton further teaches the method of claim 1, wherein the dNTP is a deoxyinosine triphosphate (dITP) analogue having the structure: (structure omitted, see applicant's Claim 2) wherein R' is a cleavable chemical group (para [0097], base analogues and derivatives, Figure 3, cleavable blocking groups, para [0189], cleavable removal).

As per Claim 3, Milton further teaches the method of claim 1, wherein the base of the dNTP is adenine, guanine, cytosine, uracil or thymine (para [0095]).

As per Claim 4, Milton further teaches the method of claim 1, 2 or 3, wherein the nucleic acid is DNA and the nucleic acid polymerase is a DNA polymerase (para [0110], DNA, para [0136], DNA polymerase).

As per Claim 9, Milton further teaches the method of claim 1 or 2, wherein the 1 carbon of the dideoxyribose is bonded to the 9 nitrogen of an inosine base (para [0097], analogues and derivatives of nucleotides).

As per Claim 13., Odedra teaches a method for determining the identity of consecutive nucleotide residues in a self-priming nucleic acid comprising: a) contacting a plurality of the nucleic acids with (i) a dideoxynucleotide triphosphate (ddNTP) analogue having the structure: (structure omitted, see applicant's Claim 13) wherein F is a fluorophore, L is a cleavable linker molecule and the base is adenine, guanine, cytosine, uracil or thymine (col 3, ln 1-20, Z can be H, Y is a cleavable linker, R2 is a fluorophore), and wherein each base has a different fluorophore attached (col 5, ln 62-65), and wherein the fluorophore attached to each type of base differs in its excitation or emission spectra from the fluorophores attached to the other types of bases (col 6, ln 1-5), but does not teach (ii) a deoxynucleotide triphosphate having the structure: (structure omitted, see applicant's Claim 13) (dNTP) analogue wherein B is a base and is adenine, guanine, cytosine, uracil, thymine or an inosine, and wherein R' is a cleavable chemical group, (iii) a nucleic acid polymerase and (iv) at least two primers each of which hybridizes with a separate nucleic acid of the plurality of nucleic acids, under conditions permitting a ddNTP analogue that is complementary to the consecutive nucleotide residue to be identified to form a phosphodiester bond with the 3' end of one of the primers and a dNTP analogue that is complementary to the consecutive nucleotide residue to be identified to form a phosphodiester bond with the 3' end of another of the primers; b) identifying the fluorophore of the ddNTP analogue which has formed the phosphodiester bond thereby identifying the identify of the consecutive nucleotide; c) cleaving the fluorophore from the ddNTP analogue which has formed the phosphodiester bond and cleaving the cleavable chemical group from the dNTP which has formed the phosphodiester bond; d) iteratively repeating steps a) through c) for each of the consecutive nucleotide residues to be identified until the final consecutive nucleotide residue is to be identified; e) repeating steps a) and b) to identify the final consecutive nucleotide residue, thereby determining the identity of each of the series of consecutive nucleotide residues in the nucleic acid. While Milton does not teach a method for determining the identity of each of a series of consecutive nucleotide residues in a nucleic acid comprising: a) contacting a plurality of the nucleic acids with (i) a dideoxynucleotide triphosphate (ddNTP) analogue having the structure: (structure omitted, see applicant's Claim 13) wherein F is a fluorophore, L is a cleavable linker molecule and the base is adenine, guanine, cytosine, uracil or thymine, and wherein each base has a different fluorophore attached, and wherein the fluorophore attached to each type of base differs in its excitation or emission spectra from the fluorophores attached to the other types of bases, Milton does further teach a deoxynucleotide triphosphate having the structure: (structure omitted, see applicant's Claim 13) (dNTP) analogue wherein B is a base and is adenine, guanine, cytosine, uracil, thymine or an inosine, and wherein R' is a cleavable chemical group (para [0147], azidomethyl group is applicant's R', para [0118], 32P label).

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Supplemental Box

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Prior Supplemental Box:

(claim 13 cont'd) (iii) a nucleic acid polymerase (para [0045]) as well as a method to use self-priming nucleic acid (para [0140], use of hairpin template DNA to serve as primer, para [0186], Figures 5 and 6). Neither Odedra nor Milton individually teach a method for determining the identity of each of a series of consecutive nucleotide residues in a nucleic acid comprising: (iv) at least two primers each of which hybridizes with a separate nucleic acid of the plurality of nucleic acids, under conditions permitting a ddNTP analogue that is complementary to the consecutive nucleotide residue to be identified to form a phosphodiester bond with the 3' end of one of the primers and a dNTP analogue that is complementary to the consecutive nucleotide residue to be identified to form a phosphodiester bond with the 3' end of another of the primers; b) identifying the fluorophore of the ddNTP analogue which has formed the phosphodiester bond thereby identifying the identify of the consecutive nucleotide; c) cleaving the fluorophore from the ddNTP analogue which has formed the phosphodiester bond and cleaving the cleavable chemical group from the dNTP which has formed the phosphodiester bond; d) iteratively repeating steps a) through c) for each of the consecutive nucleotide residues to be identified until the final consecutive nucleotide residue is to be identified; e) repeating steps a) and b) to identify the final consecutive nucleotide residue, thereby determining the identity of each of the series of consecutive nucleotide residues in the nucleic acid. However, Milton contemplates the use of a specific embodiment of his invention in Sanger or Sanger like reactions (para [0115], ddNTP approach) in which the 3' O is blocked using applicant's R' blocking group as well as having a fluorophore attached to the base (applicant has fluorophore attached to a separate, ddNTP nucleotide) while incorporating sequencing by synthesis approach (each added nucleotide is detected). In this context, it would have been obvious to one of ordinary skill in the art to apply the teachings of Odedra, namely the use of a deoxyribonucleotide having a cleavable linker attached to reporter group bound to the nitrogen base in which the 3' OH is either blocked or replaced by a H (making it a ddNTP) to the teachings of Milton, namely, the use of a deoxyribonucleotide having a cleavable 3' azidomethyl blocking group to generate the method described in Claim 1 because the utility of employing the Sanger approach without the expense of of a gel electrophoresis would be of great benefit.

As per Claim 14, Milton further teaches the method of claim 13, wherein the dNTP is a deoxyinosine triphosphate (dITP) analogue having the structure:(structure omitted, see applicant's Claim 14), wherein R' is a cleavable chemical group (para [0097], base analogues and derivatives, Figure 3, cleavable blocking groups, para [0189], cleavable removal).

As per Claim 15, Milton further teaches the method of claim 13, wherein the base of the dNTP is adenine, guanine, cytosine, uracil or thymine (para [0095]).

As per Claim 16, Milton further teaches the method of claim 13, 14 or 15, wherein the nucleic acid is DNA and the nucleic acid polymerase is a DNA polymerase (para [0110], DNA, para [0136], DNA polymerase).

As per Claim 21, Milton further teaches the method of claim 13 or 14 wherein the 1 carbon of the dideoxyribose is bonded to the 9 nitrogen of an inosine base (para [0097], analogues and derivatives of nucleotides)..

As per Claim 25 Milton furher teaches the method of claims 1 or 13 wherein the steps are performed in the order a), b), c), d), and e) (para [0141]-[0145]).

As per Claim 26, Milton further teaches the method of claims 1 or 13 wherein the steps are performed in the order a), c), b), d), and e) (para [0107]-[0109], method embodies the use of alternate detection systems, including secondary systems which conceivably be used to identify two adjoining bases after cleavage of the linker).

As per Claim 35, Odedra teaches a method for determining the identity of each of a series of consecutive nucleotide residues in a nucleic acid comprising: a) contacting a plurality of the nucleic acids with (i) at least four different dideoxynucleotide triphosphate (ddNTP) analogues, each having the structure: (structure omitted, see applicant's Claim 35) wherein F is a fluorophore, b is a base which is adenine, guanine, cytosine, uracil or thymine (col 3, ln 1-20, Z can be H, Y is a cleavable linker, R2 is a fluorophore), wherein the fluorophore attached through a linker to each type of base differs in its emission or excitation spectra from a fluorophore attached to each of the remaining types of bases, and each of the four ddNTP analogues differs from the remaining three ddNTP analogues by having a different base, wherein L is a cleavable linker molecule (col 6, ln 1-5). Milton further teaches a deoxynucleotide triphosphate having the structure: (structure omitted, see applicant's Claim 35) (dNTP) analogue wherein B is a base and is adenine, guanine, cytosine, uracil, thymine or an inosine, and wherein R' is a cleavable chemical group (para [0147], azidomethyl group is applicant's R', para [0118], 32P label), (iii) a nucleic acid polymerase (para [0045]).

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Supplemental Box

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(claim 35 cont'd) Neither Odedra nor Milton individually teach (ii) at least four deoxynucleotide triphosphate (dNTP) analogues having the structure: (structure omitted, see applicant's Claim 35) wherein B is a base and is adenine, guanine, cytosine, uracil, or thymine, and wherein R' is a cleavable chemical group, wherein each of the four dNTP analogues differs from the remaining three dNTP analogues by having a different base, (iii) a nucleic acid polymerase and (iv) a plurality of nucleic acid primers which can each hybridize with a separate one of each of the plurality of nucleic acids, under conditions permitting (a) one of the four ddNTP analogues that is complementary to the consecutive nucleotide residue to be identified to form a phosphodiester bond with the 3' end of one of the nucleic acid primers and thereby extend the primer and (b) one of the four dNTP analogues that is complementary to a consecutive nucleotide residue to be identified to form a phosphodiester. bond with the 3' end of another one of the nucleic acid primers and thereby extend that primer; b) identifying the fluorophore of the ddNTP analogue which has formed the phosphodiester bond, thereby identifying the consecutive nucleotide; c) cleaving the linker attaching the fluorophore of the ddNTP analogue which has formed the phosphodiester bond and cleaving the cleavable chemical group from the dNTP which has formed the phosphodiester bond; d) iteratively repeating steps a) through c) for each of the consecutive nucleotide residues to be identified until the final consecutive nucleotide residue is to be identified; e) repeating steps a) and b) to identify the final consecutive nucleotide residue, f) denaturing the extended primers so that they dehybridize from the plurality of nucleic acids; g) contacting the plurality of nucleic acids with (i) at least four different deoxynucleotide triphosphate (dNTP) analogues, each comprising a base chosen from adenine, thymine, cytosine, uracil, inosine, or 5nitroindole each differing from a deoxynucleotide triphosphate by having a cleavable chemical group attached to the 3' O-atom of the dNTP, (ii) a nucleic acid polymerase and (iii) a plurality of second nucleic acid primers which each separately hybridize with a separate one of the plurality of nucleic acids, under conditions permitting one of the four dNTP analogues that is complementary to the consecutive nucleotide residue to be identified to form a phosphodiester bond with the 3' end of one of the second nucleic acid primers and thereby extend that second primer; h) cleaving the chemica.l group from the 3' O-atom of the dNTP analogue which has formed the phosphodiester bond so as to thereby permit incorporation of a further dNTP analogue into the extended second nucleic acid primer; i) iteratively repeating steps g) and h) until the second primer is extended up to and including a residue corresponding to the final consecutive nucleotide residue identified in step e); j) contacting the plurality of extended second primers with (i) at least four different dideoxynucleotide triphosphate (ddNTP) analogues each having the structure: (structure omitted, see applicant's Claim 35) wherein F is a fluorophore, b is a base which is adenine, guanine, cytosine, uracil or thymine, wherein the fluorophore attached through a linker to each type of base differs in its emission or excitation spectra from a fluorophora attached to each of the remaining types of bases, and each of the four ddNTP analogues differs from the remaining three ddNTP analogues by having a different base, wherein L is a cleavable linker molecule, (ii) at least four deoxynucleotide triphosphate (dNTP) analogues having the structure: (structure omitted, see applicant's Claim 35) wherein B is a base and is adenine, guanine, cytosine, uracil, or thymine, and wherein R' is a cleavable chemical group, wherein each of the four dNTP analogues differs from the remaining three dNTP analogues by having a different base, and (iii) a nucleic acid polymerase, under conditions permitting (a) one of the four ddNTP analogues that is complementary to the next consecutive nucleotide residue to be identified to form a phosphodiester bond with the 3' end of one of the extended second nucleic acid primers and thereby extend the second primer and (b) one of the four dNTP analogues that is complementary to a consecutive nucleotide residue to be identified to form a phosphodiester bond with the 3' end of another one of the extended second nucleic acid primers and thereby extend that second primer; k) identifying the fluorophore of the ddNTP analogue which has formed the phosphodiester bond, thereby identifying the consecutive nucleotide; 1) cleaving the linker attaching the fluorophore of the ddNTP analogue which has formed the phosphodiester bond and cleaving the cleavable chemical group from the dNTP which has formed the phosphodiester bond; m) iteratively repeating steps j) through 1) for each of the consecutive nucleotide residues to be identified until the final consecutive nucleotide residue is to be identified; n) repeating steps j) and k) to identify the final consecutive nucleotide residue, so as to thereby determine the identity of each of the series of consecutive nucleotide residues in the nucleic acid. However, Milton contemplates the use of a specific embodiment of his invention in Sanger or Sanger like reactions (para [0115], ddNTP approach) in which the 3' O is blocked using applicant's R' blocking group as well as having a fluorophore attached to the base (applicant has fluorophore attached to a separate, ddNTP nucleotide) while incorporating sequencing by synthesis approach (each added nucleotide is detected). In this context, it would have been obvious to one of ordinary skill in the art to apply the teachings of Odedra, namely the use of a deoxyribonucleotide having a cleavable linker attached to reporter group bound to the nitrogen base in which the 3' OH is either blocked or replaced by a H (making it a ddNTP) to the teachings of Milton, namely, the use of a deoxyribonucleotide having a cleavable 3' azidomethyl blocking group to generate the method described in Claim 35 because the utility of employing the Sanger approach without the expense of of a gel electrophoresis would be of great benefit.

As per Claim 36, Milton does not teach the method of claim 35, wherein the linker in each of step a) and j) independently each comprise thestructure: (structure omitted, see applicant's Claim 36) wherein a represents a point of attachment to the base and b represents a point of attachment to the fluorophore, and wherein R is a cleavable chemical group. Milton, however, does teach cleavable groups utilizing the same functional groups as in Claim 36 (para [006], Figure 3), and which would render the limitation of claim 36 obvious.

As per Claim 37, Milton further teaches the method of claim 35 or 36, wherein a linker is cleaved by contacting the linker with tris(2-carboxyethyl) phosphine (para [0077])

As per Claim 38, Milton further teaches the method of claim 35, wherein one or more linkers are photocleavable or chemically cleavable (para [0042], para [0123], photocleavable, para [0077], chemical).

As per Claim 39, Milton further teaches the method of claim 35, wherein one or more chemical groups are photocleavable or chemically cleavable (para [0123], photocleavable groups, para [0189], chemical cleavage of azidomethy; blocking group using TCEP).

As per Claim 40, Milton further teaches the method of claim 35 or 36, wherein R in the structures set forth in steps a) and or j) is independently chosen from a -N3 group or an allyl group (para [0088], N3, para [0024], allyl group).

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As per Claim 41, Milton further teaches the method of claim 35 or 36, wherein the cleavable chemical group in step g) is independently chosen from a -N3 group or an allyl group (para [0088], N3, para [0024], allyl group).

As per Claim 42, Odedra teaches a method for determining the identity of each of a series of consecutive nucleotide residues in a nucleic acid comprising: a) contacting a plurality of the nucleic acids with (i) at least four different dideoxynucleotide triphosphate (ddNTP) analogues, each having the structure: (structure omitted, see applicant's Claim 42) wherein F is a fluorophore, b is a base which is adenine, guanine, cytosine, uracil or thymine (col 3, ln 1-20, Z can be H, Y is a cleavable linker, R2 is a fluorophore), wherein the fluorophore attached through a linker to each type of base differs in its emission or excitation spectra from a fluorophore attached to each of the remaining types of bases, and each of the four ddNTP analogues differs from the remaining three ddNTP analogues by having a different base, wherein L is a cleavable linker molecule (col 6, ln 1-5). Milton further teaches a deoxynucleotide triphosphate having the structure: (structure omitted, see applicant's Claim 35) (dNTP) analogue wherein B is a base and is adenine, guanine, cytosine, uracil, thymine or an inosine, and wherein R' is a cleavable chemical group (para [0147], azidomethyl group is applicant's R', para [0118], 32P label), (iii) a nucleic acid polymerase (para [0045]). Neither Odedra nor Milton individually teach ii) at least four deoxynucleotide triphosphate (dNTP) analogues having the structure: (structure omitted, see applicant's Claim 42) wherein B is a base and is adenine, guanine, cytosine, uracil, or a thymine, and wherein R' is a cleavable chemical group, wherein each of the four dNTP analogues differs from the remaining three dNTP analogues by having a different base, (iii) a nucleic acid polymerase and (iv) a plurality of nucleic acid primers which can each hybridize with a separate one of each of the plurality of nucleic acids, under conditions permitting (a) one of the four ddNTP analogues that is complementary to the consecutive nucleotide residue to be identified to form a phosphodiester bond with the 3' end of one of the nucleic acid primers and thereby extend the primer and (b) one of the four dNTP analogues that is complementary to a consecutive nucleotide residue to be identified to form a phosphodiester bond with the 3' end of another one of the nucleic acid primers and thereby extend that primer; b) identifying the fluorophore of the ddNTP analogue which has formed the phosphodiester bond, thereby identifying the consecutive nucleotide; c) cleaving the linker attaching the fluorophore of the ddNTP analogue which has formed the phosphodiester bond and cleaving the cleavable chemical group from the dNTP which has formed the phosphodiester bond; d) iteratively repeating steps a) through c) for each of the consecutive nucleotide residues to be identified until the final consecutive nucleotide residue is to be identified; e) repeating steps a) and b) to identify the final consecutive nucleotide residue, f) denaturing the extended primers so as to dehybridize them from the plurality of nucleic acids; g) contacting the nucleic acids with (i) three different types of deoxynucleotide triphosphate, (ii) a nucleic acid polymerase and (iii) a second plurality of nucleic acid primers which each hybridize with a separate one of the plurality of nucleic acids, under conditions permitting one of the three dNTP analogues that is complementary to the consecutive nucleotide residue to be identified to form a phosphodiester bond with the 3' end of the second nucleic acid primer and thereby extend the second nucleic acid primer; h) contacting the nucleic acid with (i) three different types of deoxynucleotide triphosphate, wherein at least one of the types of deoxynucleotide triphosphate is not used in step g) , under conditions permitting one of the three dNTP that is complementary to the consecutive nucleotide residue to be identified to form a phosphodiester bond with the 3' end of the extended second nucleic acid primer and thereby further extend the second nucleic acid primer; i) repeating steps g) and h) until the second nucleic acid primer is extended up to and including a residue corresponding to the final consecutive nucleotide residue identified in step e) j) contacting the plurality of extended second primers with (i) at least four different dideoxynucleotide triphosphate (ddNTP) analogues, each having the structure: (structure omitted, see applicant's Claim 42) wherein F is a fluorophore, b is a base which is adenine, guanine, cytosine, uracil or thymine, wherein the fluorophore attached through a linker to each type of base differs in its emission or excitation spectra from a fluorophore attached to each of the remaining types of bases, and each of the four ddNTP analogues differs from the remaining three ddNTP analogues by having a different base, wherein L is a cleavable linker molecule, (ii) at least four deoxynucleotide triphosphate (dNTP) analogues having the structure: (structure omitted, see applicant's Claim 42) wherein B is a base and is adenine, guanine, cytosine, uracil, or a thymine, and wherein R' is a cleavable chemical group, wherein each of the four dNTP analogues differs from the remaining three dNTP analogues by having a different base, and (iii) a nucleic acid polymerase, under conditions permitting (a) one of the four ddNTP analogues that is complementary to the consecutive nucleotide residue to be identified to form a phosphodiester bond with the 3' end of one of the extended second nucleic acid primers and thereby extend the second primer and (b) one of the four dNTP analogues that is complementary to a consecutive nucleotide residue to be identified to form a phosphodiester bond with the 3' end of another one of the extended second nucleic acid primers and thereby extend that second primer; k) identifying the fluorophore of the ddNTP analogue which has formed the phosphodiester bond, thereby identifying the consecutive nucleotide; 1) cleaving the linker attaching the fluorophore of the ddNTP analogue which has formed the phosphodiester bond and cleaving the cleavable chemical group from the dNTP which has formed the phosphodiester bond; m) iteratively repeating steps j) through 1) for each of the consecutive nucleotide residues to be identified until the final consecutive nucleotide residue is to be identified; n) repeating steps j) and k) to identify the final consecutive nucleotide residue, so as to thereby determine the identity of each of the series of consecutive nucleotide residues in the nucleic acid. However, Milton contemplates the use of a specific embodiment of his invention in Sanger or Sanger like reactions (para [0115], ddNTP approach) in which the 3' O is blocked using applicant's R' blocking group as well as having a fluorophore attached to the base (applicant has fluorophore attached to a separate, ddNTP nucleotide) while incorporating sequencing by synthesis approach (each added nucleotide is detected). In this context, it would have been obvious to one of ordinary skill in the art to apply the teachings of Odedra, namely the use of a deoxyribonucleotide having a cleavable linker attached to reporter group bound to the nitrogen base in which the 3' OH is either blocked or replaced by a H (making it a ddNTP) to the teachings of Milton, namely, the use of a deoxyribonucleotide having a cleavable 3' azidomethyl blocking group to generate the method described in Claim 43 because the utility of employing the Sanger approach without the expense of of a gel electrophoresis would be of great benefit.

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As per Claim 43, Milton further teaches the method of claim 42, wherein in steps g) and h) the three types of dNTPs are chosen from the group dATP, dCTP, dGTP and dTTP (para [0095]).

As per Claim 44, Milton further teaches the method of claim 42, wherein the linker in each of step a) and j) independently each comprise the structure: (structure omitted, see applicant's Claim 43), or the structure: (structure omitted, see applicant's Claim 43) wherein a represents a point of attachment to the base and b represents a point of attachment to the fl uorophore, and wherein R is a cleavable chemical group. Milton teaches cleavable groups utilizing the same functional groups as in Claim 36 (para [006], Figure 3).

As per Claim 45, Milton further teaches the method of claim 42 or 44, wherein a linker is cleaved by contacting the linker with tris(2carboxyethyl) phosphine (para [0077]).

As per Claim 46, Milton further teaches the method of claim 42, wherein one or more linkers are photocleavable or chemically cleavable (para [0042], para [0123], photocleavable, para [0077], chemical).

As per Claim 47, Milton further teaches the method of claim 42, wherein one or more chemical groups are photocleavable or chemically cleavable (para [0123], photocleavable groups, para [0189], chemical cleavage of azidomethy; blocking group using TCEP).

As per Claim 48, Milton further teaches the method of claim 42 or 44, wherein R .in the structures set forth in steps a) and or j) is independently chosen from a -N3 group or an allyl group (para [0088], N3, para [0024], allyl group).

As per Claim 49, Milton further teaches the method of claim 42 or 44, wherein the cleavable chemical group in step g) is independently chosen from a -N3 group or an allyl group (para [0088], N3, para [0024], allyl group).

As per Claim 50, Odedra teaches a method for determining the identity of each of a series of consecutive nucleotide residues in a nucleic acid comprising: a) contacting a plurality of the nucleic acids with (I) at least four different dideoxynucleotide triphosphate (ddNTP) analogues, each having the structure: (structure omitted, see applicant's Claim 50), wherein F is a f1uorophore, b is a base which is adenine, guanine, cytosine, uracil or thymine (col 3, ln 1-20, Z can be H, Y is a cleavable linker, R2 is a fluorophore), wherein the fluorophore attached through a linker to each type of base differs in its emission or excitation spectra from a fluorophore attached to each of the remaining types of bases, and each of the four ddNTP analogues differs from the remaining three ddNTP analogues by having a different base, wherein L is a cleavable linker molecule (col 6, ln 1-5). Milton further teaches a deoxynucleotide triphosphate having the structure: (structure omitted, see applicant's Claim 35) (dNTP) analogue wherein B is a base and is adenine, guanine, cytosine, uracil, thymine or an inosine, and wherein R' is a cleavable chemical group (para [0147], azidomethyl group is applicant's R', para [0118], 32P label), (iii) a nucleic acid polymerase (para [0045]).

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(claim 50 cont'd) Neither Odedra nor Milton individually teach (ii) at least four deoxynucleotide triphosphate (dNTP) analogues having the structure: (structure omitted, see applicant's Claim 50), wherein B is a base and is adenine, guanine, cytosine, uracil, or a thymine, and wherein R' is a cleavable chemical group, wherein each of the four dNTP analogues differs from the remaining three dNTP analogues by having a different base, (iii) a nucleic acid polymerase and (iv) a plurality of nucleic acid primers which can each hybridize with a separate one of each of the plurality of nucleic acids, under conditions permitting (a) one of the four ddNTP analogues that is complementary to the consecutive nucleotide residue to be identified to form a phosphodiester bond with the 3' end of one of the nucleic acid primers and thereby extend the primer and (b) one of the four dNTP analogues that is complementary to a consecutive nucleotide residue to be identified to form a. phosphodiester bond with the 3' end of another one of the nucleic acid primers and thereby extend that primer; b) identifying the fluorophore of the ddNTP analogue which has formed the phosphodiester bond, thereby identifying the consecutive nucleotide; c) cleaving the linker attaching the fluorophore of the ddNTP analogue which has formed the phosphodiester bond and cleaving the cleavable chemical group from the dNTP which has formed the phosphodiester bond; d) iteratively repeating steps a) through c) for each of the consecutive nucleotide residues to be identified until the final consecutive nucleotide residue is to be identified; e) repeating steps a) and b) to identify the final consecutive nucleotide residue, f) denaturing the extended primers so as to dehybridize them from the plurality of nucleic acids; g) contacting the nucleic acid with (i) three different types of deoxynucleotide triphosphates, (ii) a deoxynucleotide triphosphate analogue, differing from a deoxynucleotide triphosphate by having a cleavable chemical group attached to the 3' O-atom of the dNTP analogue and differing from the three different types of deoxynucleotide triphosphates by having a different base therefrom, (iii) a nucleic acid polymerase and (iv) a second nucleic acid primer which hybridizes with the nucleic acid, under conditions permitting one of the three dNTPs or the dNTP analogue that is complementary to the consecutive nucleotide residue to be identified to form a phosphodiester bond with the 3' end of one of the second nucleic acid primers and thereby extend that second nucleic acid primer; h) cleaving the cleavable chemical group from the 3'-O- atom group; i) repeating steps g) and h) until the second nucleic acid primer is extended up to and including a residue corresponding to the final consecutive nucleotide residue identified in step e) j) contacting the plurality of extended second primers with (i) at least four different dideoxynucleotide triphosphate (ddNTP) analogues, each having the structure: (structure omitted, see applicant's Claim 50), wherein F is a fluorophore, b is a base which is adenine, guanine, cytosine, uracil or thymine, wherein the fluorophore attached through a linker to each type of base differs in its emission or excitation spectra from a fluorophore attached to each of the remaining types of bases, and each of the four ddNTP analogues differs from the remaining three ddNTP analogues by having a different base, wherein L is a cleavable linker molecule, (ii) at least four deoxynucleotide triphosphate (dNTP) analogues having the structure: (structure omitted, see applicant's Claim 50), wherein B is a base and is adenine, guanine, cytosine, uracil, or thymine, and wherein R' is a cleavable chemical group, wherein each of the four dNTP analogues differs from the remaining three dNTP analogues by having a different base, and (iii) a nucleic acid polymerase, under conditions permitting (a) one of the four ddNTP analogues that is complementary to the consecutive nucleotide residue to be identified to form a phosphodiester bond with the 3' end of one of the extended second nucleic acid primers and thereby extend the second primer and (b) one of the four dNTP analogues that is complementary to a consecutive nucleotide residue to be identified to form a phosphodiester bond with the 3' end of another one of the extended second nucleic acid primers and thereby extend that second primer; k) identifying the fluorophore of the ddNTP analogue which has formed the phosphodiester bond, thereby identifying the consecutive nucleotide; 1) cleaving the linker attaching the fluorophore of the ddNTP analogue which has formed the phosphodiester bond and cleaving the cleavable chemical group from the dNTP which has formed the phosphodiester bond; m) iteratively repeating steps j) through h) for each of the consecutive nucleotide residues to be identified until the final consecutive nucleotide residue is to be identified; n) repeating steps j) and k) to identify the final consecutive nucleotide residue, so as to thereby determine the identity of each of the series of consecutive nucleotide residues in the nucleic acid. However, Milton contemplates the use of a specific embodiment of his invention in Sanger or Sanger like reactions (para [0115], ddNTP approach) in which the 3' O is blocked using applicant's R' blocking group as well as having a fluorophore attached to the base (applicant has fluorophore attached to a separate, ddNTP nucleotide) while incorporating sequencing by synthesis approach (each added nucleotide is detected). In this context, it would have been obvious to one of ordinary skill in the art to apply the teachings of Odedra, namely the use of a deoxyribonucleotide having a cleavable linker attached to reporter group bound to the nitrogen base in which the 3' OH is either blocked or replaced by a H (making it a ddNTP) to the teachings of Milton, namely, the use of a deoxyribonucleotide having a cleavable 3' azidomethyl blocking group to generate the method described in Claim 51 because the utility of employing the Sanger approach without the expense of of a gel electrophoresis would be of great benefit.

As per Claim 51, Milton further teaches the method of claim 50, wherein in step c) the three types of dNTPs are chosen from the group dATP, dCTP, dGTP and dTTP. (para [0095]).

As per Claim 52, Milton does teach the method of claim 50, wherein the linker in each of step a) and f) independently each comprise thestructure: (structure omitted, see applicant's Claim 52), wherein a represents a point of attachment to the base and b represents a point of attachment to the fluorophore, and wherein R is a cleavable chemical group. However, Milton does teach cleavable groups utilizing the same functional groups as in Claim 36. (para [006], Figure 3), which would render this claim limitation obvious.

As per Claim 53, Milton further teaches the method of claim 50 or 52, wherein a linker is cleaved by contacting the linker with tris(2-carboxyethyl) phosphine (para [0077]).

As per Claim 54, Milton further teaches the method of claim 50, wherein one or more linkers are photocleavable or chemically cleavable (para [0042], para [0123], photocleavable, para [0077], chemical).

As per Claim 55, Milton further teaches the method of claim 50, wherein one or more chemical groups are photocleavable or chemically cleavable (para [0123], photocleavable groups, para [0189], chemical cleavage of azidomethy; blocking group using TCEP).

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WRITTEN OPINION OF THE
INTERNATIONAL SEARCHING AUTHORITY

International application No.
PCT/US 08/11913

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As per Claim 56, Milton further teaches the method of claim 50 or 52, wherein R in the structures set forth in steps a) and or f) is independently chosen from a -N3 group or an allyl group. (para [0088], N3, para [0024], allyl group).

As per Claim 57, Milton further teaches the method of claim 50 or 52, wherein the cleavable chemical group in step f) is independently chosen from a -N3 group or an allyl group. (para [0088], N3, para [0024], allyl group).

As per Claim 58, Milton furhter teaches the method of claim 35, 42 or 50, wherein the first and second plurality of primers have the same sequence (para [0140], target DNA forms a hairpin, in which part of it serves as a primer and the other part as the DNA to be sequenced).

As per Claim 59, Milton further teaches the method of claim 35, 42 or 50, wherein one or more washing steps are performed in between one or more of the steps set forth (para [0143] washing step).

Claims 1-4, 9, 13-16, 21, 25-26 and 28-59 have industrial applicability as defined by PCT Article 33(4) because the subject matter can be made or used in industry.